Neurobiology

LS.5.135
The effect of kainic acid on hippocampal dendritic spine morphology and motility at the early and late stages of brain development (Electron and multiphoton laser-scanning microscope study)

T. Bikashvili[1], T. Lordkipanidze[1,2]

[1]I.Beritashvili Center of Experimental Biomedicine, Neuroanatomy, Tbilisi, Georgia
[2]Ilia State University, Institute of Chemical Biology, Tbilisi, Georgia

tamarabikashvili@rambler.ru

Dendrites and spines undergo dynamic changes in physiological conditions, such as learning and memory, and in pathological conditions, such as epilepsy [1, 2]. Abnormalities in dendritic spines have commonly been observed in brain specimens from epilepsy patients and animal models of epilepsy [3, 4]. However, the functional implications and clinical consequences of this dendritic pathology for epilepsy are uncertain. Motility of dendritic spines and axonal filopodia has been recently discovered by the advanced imaging techniques, and remains to a large degree an exciting phenomenology in search of function [5]. We demonstrate the effect of kainic acid (KA), which is a structural analog of glutamate, on dendritic spine morphology and motility in hippocampal CA1 area at the different stages of brain development. We used Electron Microscopy and Two-photon Microscopy in our experiments.

In order to reveal the changes that take place in spine and filopodial motility in the epileptic model of brain, time-lapse imaging of acute hippocampal slices treated with various concentrations of KA after different incubation time points was performed. The effects of KA exposure were tested on the slices from young (postnatal day (P7–P10) and adolescent (P28–P30) Thy1-YFPH transgenic mice in which pyramidal hippocampal neurons express green fluorescence (Figure 1). Slices were treated with either 50 µM or 100 µM of KA, for either 30 or 100 min. Three-dimensional reconstruction of spines showed the variability in their morphology: stubby, thin, mushroom and ramified. The results obtained in our experiments show diverse effects of KA in 2 different age groups. According to our results, 100 µM/100 min KA treatment increases spine motility at early stage of brain development (P10) by 41.5%, while in P30 mice spine motility is increased only by 3%. Our findings also indicate that effect of KA on hippocampal dendritic spine motility is predominantly time- rather than concentration-dependent.
Imaging was conducted using multiphoton laser-scanning microscope (Movable Objective Microscope, Sutter) with a Ti-Saphire laser (Chameon Vision II, Coherent) at 920 nm. High-resolution imaging was performed with a long working distance, dipping objective 60X, N.A.1. Images were collected every 30s for a period of 15 min at a digital zoom of 4 (yielding a pixel size of 0.08 × 0.08 µm). At each time point, seven to ten focal planes 0.5 µm apart were collected. Slices were perfused with oxygenated artificial cerebral spinal fluid (ACSF) at 35-37^{0}C, the imaging chamber was kept at 35-370C (Warner Instruments), and the slices were held in place using a platinum and nylon harp (Figure 2, Figure 3).
Spine motility was quantified using “Motility Index” [5]. The motility index measures the overall displacement of a process. We first measure the area of a process at seven time points that differ the most from each other in a single time-lapse movie, then subtract the smallest area from the total projected or accumulated area and divide by the the average area.

Data were analyzed using one-way ANOVA test. In the case of significant effect planned comparisons were carried out using student t-test. The level of significance was set as p<0.05. All data are presented as a mean +/- standard error of the mean (SEM).

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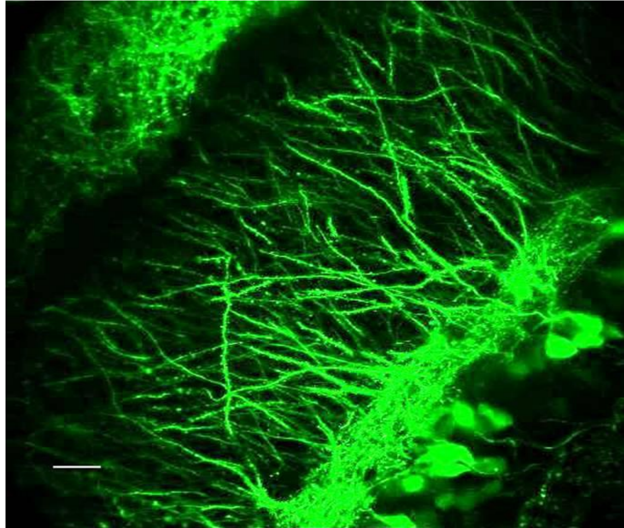

Figure 1. Low magnification (40x objective; zoom 2) image of the dendrites in the hippocampal CA1 area from Thy1-YFPH mouse where all pyramidal neurons express YFP. Scale bar = 22μm.

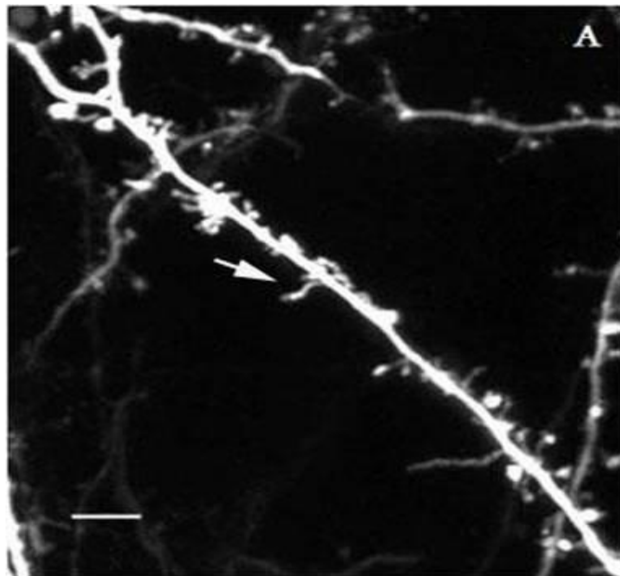

Figure 2. Dendrite with spines from P10 mice. High magnification (60x objective; zoom 4). Time-lapse imaging. Arrow indicates the spine with motility index 2.0. Scale bar = 4 μm.

Figure 3. The most different 7 z- stacks of motile spine.